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REVIEW

Structures, Biosynthesis, and Functions of Gangliosides-an Overview

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Abstract: Gangliosides are sialic acid-containing glycosphingolipids that are most abundant in the nervous system. Heterogeneity and diversity of the structures in their carbohydrate chains are characteristic hallmarks of these lipids; so far, 188 gangliosides with different carbohydrate structures have been identified in vertebrates. The molecular structural complexity increases manifold if one considers heterogeneity in the lipophilic components. The expression levels and patterns of brain gangliosides are known to change drastically during development. In cells, gangliosides are primarily, but not exclusively, localized in the outer leaflets of plasma membranes and are integral components of cell surface microdomains with sphingomyelin and cholesterol from which they participate in cell-cell recognition, adhesion, and signal transduction. In this brief review, we discuss the structures, metabolism and functions of gangliosides.

Key words: glycosyltransferase, glycosphingolipid, knockout mouse, stem cell, neural stem cell

1 Structures and metabolism of gangliosides

Glycolipids are biomolecules containing one or more carbohydrate residues linked to a hydrophobic lipid moiety through a glycosidic linkage. Glycolipids containing either a sphingoid or a ceramide as the hydrophobic lipid moiety are referred to as glycosphingolipids. Glycosphingolipids possess highly heterogeneous and diverse molecular structures in their carbohydrate chains and the lipid moieties. Based on their basic carbohydrate structures, glycosphingolipids are classified into the following series, namely, ganglio-, isoganglio-, lacto-, neolacto-, lactoganglio-, globo-, isoglobo-, muco-, gala-, neogala-, mollu-, arthro-, schisto- and spirometo-series (Table 1). Acidic glycosphingolipids containing one or more sialic acid (N-acetylneuraminic acid or N-glycolylneuraminic acid) residue (s) in their carbohydrate moiety are especially referred to as gangliosides. Figure 1 depicts a common brain ganglioside, GM1. As of 2004, 188 gangliosides have been identified in vertebrate tissues[1].

Figure 2 shows the structures and metabolic pathways of ganglio-series gangliosides. Ganglio-series glycosphingolipids having 0, 1, 2 and 3 sialic acid residue (s) linked to the inner galactose residue are classified into asialo-, a-, b- and c-series gangliosides, respectively. In addition, gangliosides having sialic acid residue (s) linked to the inner N-acetylgalactosamine residue, such as GT1aα originally reported as GTx[2], are classified as α-series gangliosides.

Glycosphingolipids including these gangliosides are primarily synthesized in the endoplasmic reticulum and further modified in the Golgi apparatus by sequential addition of additional carbohydrate moieties[3] to an existing acceptor lipid molecule. The reaction is catalyzed by a series of specific glycosyltransferases. With the exception of GM4, which is derived from galactosylceramide (GalCer), most gangliosides are synthesized from lactosylceramide (LacCer). First, a simple ganglioside, GM3, is synthesized by addition of a sialic acid to LacCer by CMP-sialic acid: LacCer α2-3 sialyltransferase (ST-I or GM3 synthase). GD3 and GT3 are synthesized by sequential addition of sialic acids to GM3 and GD3 by CMP-sialic acid: GM3 α2-8 sialyltransferase (ST-II or GD3 synthase) and CMP-sialic acid: GD3 α2-8 sialyltransferase (ST-III or GT3 synthase), respectively. GM3, GD3 and GT3 further serve as precursors of more complex gangliosides belonging to the a-, b- and c-series, respectively. Elaboration of these simple gangliosides to complex gangliosides is catalyzed by UDP-GalNAc: LacCer/GM3/GD3/GT3 β1-4 N-acetylgalactosaminyltransferase (GalNAcT or GA2/GM2/GD2/GT2 synthase),

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Table 1 Carbohydrate structures of glycosphingolipids.

Series	Basic structure	Abbreviation
Globo	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1'Cer	Gb
Isoglobo	GalNAcβ1-3Galα1-3Galβ1-4Glcβ1-1'Cer	iGb
Ganglio	Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-1'Cer	Gg
Isoganglio	Galβ1-3GalNAcβ1-3Galβ1-4Glcβ1-1'Cer	iGg
Lacto	Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1'Cer	Lc
Neolacto	Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1'Cer	nLc
Lactoganglio	GalNAcβ1-4(GlcNAcβ1-3)Galβ1-4Glcβ1-1'Cer	LcGg
Muco	Galβ1-4Galβ1-4Glcβ1-1'Cer	Mc
Gala	Galα1-4Galβ1-1'Cer	Ga
Neogala	Galβ1-6Galβ1-6Galβ1-1'Cer	
Mollu	GlcNAcβ1-2Manα1-3Manβ1-4Glcβ1-1'Cer	Mu
Arthro	GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glcβ1-1'Cer	At
Schisto	GalNAcβ1-4Glcβ1-1'Cer	
Spirometo	Galβ1-4Glcβ1-3Galβ1-1'Cer	

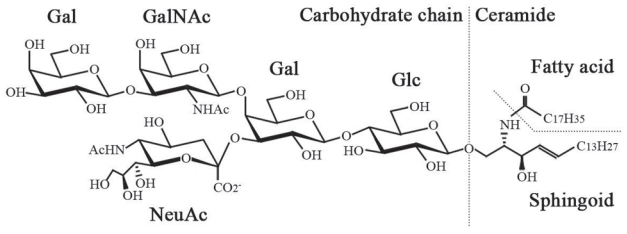

Fig. 1 Structure of GM1 ganglioside.

UDP-Gal: GA2/GM2/GD2/GT2 β1-3 galactosyltransferase (GalT-II or GA1/GM1/GD1b/GT1c synthase), CMP-sialic acid: GA1/GM1/GD1b/GT1c α2-3 sialyltransferase (ST-IV or GM1b/GD1a/GT1b/GQ1c synthase), and CMP-sialic acid: GM1b/GD1a/GT1b/GQ1c α2-8 sialyltransferase (ST-V or GD1α/GT1aα/GQ1bα/GP1cα). Asialo-series gangliosides are also synthesized from LacCer by these glycosyltransferases along a different pathway.

Interestingly, the expression levels and patterns of ganglio-series gangliosides undergo dramatic changes during brain development[4, 5]. For instance, in human and rodent embryonic brains, the predominant gangliosides are simple GM3 and GD3. As the brain develops, the expression of these simple gangliosides is down-regulated with concomitant up-regulation of complex gangliosides such as GM1, GD1a, GD1b and GT1b. This change in expression levels and patterns of gangliosides can be largely attributed to the developmental change of the expression levels and patterns of ganglioside synthases (glycosyltransferases)[6, 7] that are spatiotemporally regulated, both at the transcriptional and post-translational levels, by multiple systems, including transcription factors[7] and probably epigenetic modifications[8].

2 Functions of gangliosides

Gangliosides are ubiquitously found in tissues and body fluids, and are more abundantly expressed in the nervous system[9]. In cells, gangliosides are primarily, but not exclusively, localized in the outer leaflets of plasma membranes. On the cell surface, gangliosides are involved in cell-cell recognition and adhesion and signal transduction within specific cell surface microdomains, termed caveolae[10], lipid rafts[11], or glycosphingolipid-enriched microdomains[12], with other membrane components such as sphingomyelin and cholesterol. Evidence is accumulating that gangliosides are colocalized in the microdomain structures with signaling molecules and adhesion molecules. In addition to cell plasma membranes, gangliosides have been shown to be present on nuclear membranes, and they have recently been proposed to play important roles in modulating intracellular and intranuclear calcium homeostasis and the ensuing cellular functions[13].

The biological importance of gangliosides has been revealed by analyses of genetically engineered mice deficient in ganglioside synthases (Table 2). In histological studies of ST-I knockout mice, selective degeneration of the organ of Corti (sensory organ of hearing in the cochlea) occurs, coincidently with the onset of hearing loss. The loss of GM3 in this mutant may contribute to complete deafness[14]. This observation implicates a role for GM3 ganglioside and its derivatives in the functional maturation of the cochlea during early development. Recently, it has been reported that the ST-I knockout mice exhibit a phenotype resembling attention-deficit hyperactivity disorder[15], thus indicating a novel role of glycosphingolipids for maintaining neuropsychological balance.

ST-II knockout mice, deficient in b- and c-series ganglio-

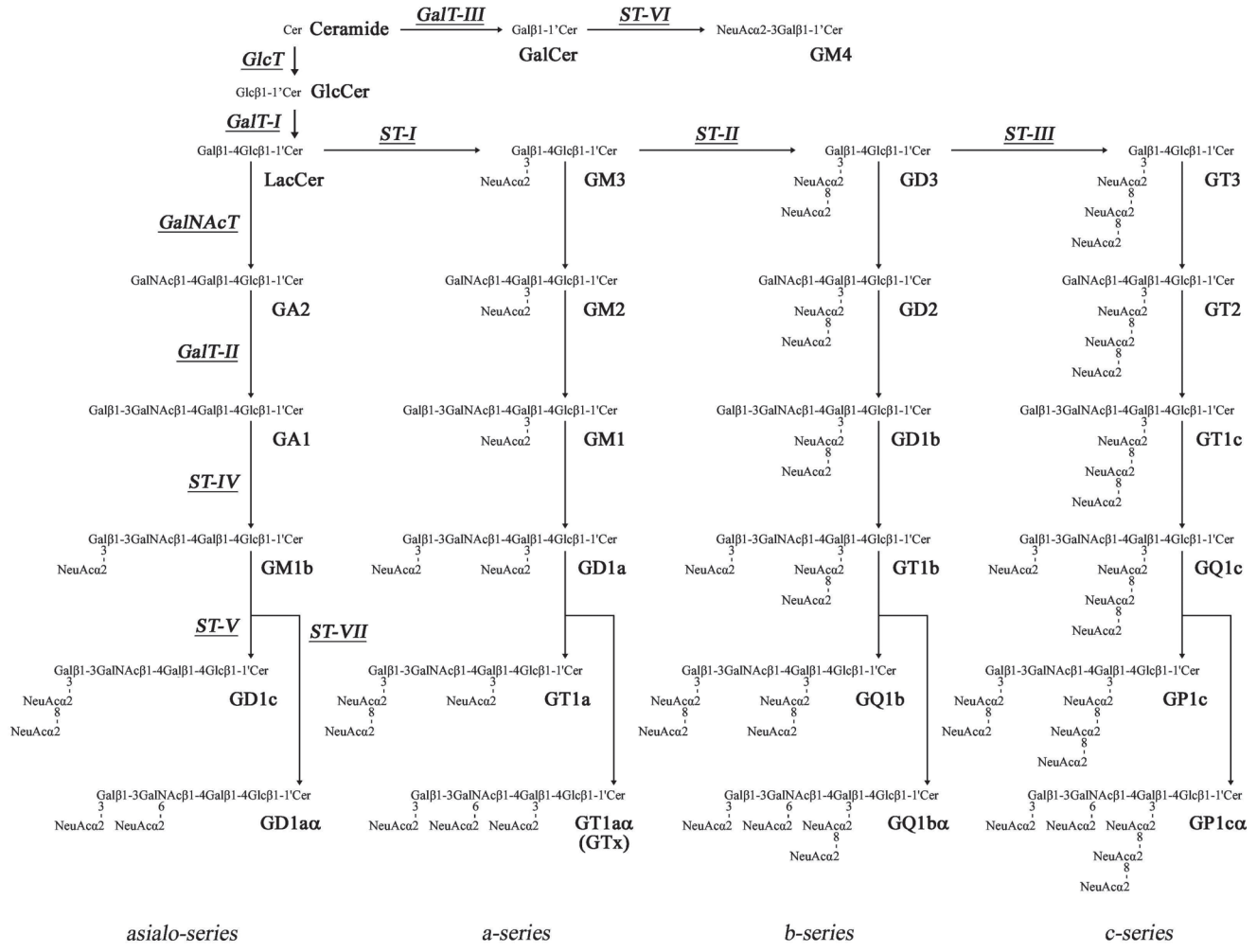

Fig. 2 Structures and biosynthetic pathways of ganglio-series gangliosides. The nomenclature for gangliosides and the components are based on those of Svennerholm[53] and IUPAC-IUBMB Joint Commission on Biochemical Nomenclature[54], respectively. Glycosyltransferases catalyzing the synthesis of glycosphingolipids, including gangliosides, are underlined. GD1α, GT1aα, GQ1bα, and GP1cα are classified as belonging to the α-series gangliosides.

sides, exhibit intact nervous tissue morphology; however, the regenerative ability of injured hypoglossal nerve in these mice is found to be severely impaired[16].

Mice lacking complex hexosamine-containing "brain-type" gangliosides, such as GM1, GD1a, GD1b and GT1b, caused by GalNAcT gene disruption show apparently normal histogenesis of brain and gross behavior[17], but impaired motor coordination in older animals[18]. Interestingly, the nerve conduction velocity is significantly lower in GalNAcT knockout mice than in the wild type, as demonstrated by analyses of evoked potentials of contralateral S1 somatosensory cortex after stimulating the peripheral tibial nerve at the Achilles tendon[17], suggesting the involvement of complex gangliosides in neural functions, such as neuronal transmission, or in structural maintenance of the nervous system. The latter hypothesis has been supported by detailed morphological analyses with electron microscopy of the GalNAcT knockout mice: those animals revealed decreased myelination and axonal degeneration in sciatic nerves and demyelination in optic nerves[19, 20], as well as neural degeneration, glial enlargement and synaptic remodeling in the dorsal horn of the spinal cord and dorsal root ganglia, especially in the sensory nerve system[20].

When GalNAcT and ST-II genes are disrupted simultaneously, the double knockout mice express primarily GM3 with no "brain-type" gangliosides. These mice exhibit weight loss, progressive motor and sensory dysfunctions and deterioration in spatial learning and memory with aging[21, 22]. Additionally, the responses to treatment with oxotremorine, an agonist of muscarinic acetylcholine re-

Table 2 Phenotypes of ganglioside synthase-KO mice.

Disrupted gene	Gangliosides expressed in brain*	Phenotypes of the nervous system
ST-I	GM1b, GD1α[14]	(Viable)
		Complete hearing loss[14]
		Degeneration of the sensory organ of hearing in cochlea[14]
		Attention-deficit hyperactivity disorder-like behavior[15]
ST-II	GM1, GD1a[16, 23, 25]	(Viable)
	GM3, GD1a in embryo[16]	Impaired regeneration of the lesioned hypoglossal nerve[16]
GalNAcT	GM3, GD3[17, 23, 25]	(Viable)
		Decreased myelination and axonal degeneration in CNS/PNS[19]
		Demyelination in PNS[19]
		Reduction in neural conduction velocity from the tibial nerve to the somatosensory cortex[17]
		Sensory nerve-dominant nerve degeneration and synaptic remodeling[20]
		Symptoms of Parkinson's disease: loss of dopaminergic neurons of the substantia nigra pars compacta, and aggregation of α-synuclein[55]
ST-I/GalNAcT	(ganglioside deficient)[26]	(Viable; death soon after weaning)[26]
		Axonal degeneration and perturbed axon-glia interaction in CNS[26]
ST-II/GalNAcT	GM3[23-25]	(Viable; shortened life span)[25]
		Sudden death in response to lethal sound-induced seizures[25]
		Neurodegeneration by dysfunction of complement systems and inflamation[23, 24]
		GEM/raft transfiguration, complement activation, local inflammation[23, 24]
		Progressive dysfunction of motor coordination, marked deterioration in memory and learning[21, 22]
		Suppressed function of muscarinic type acetylcholine receptors[22]

*In wildtype mice, the predominant gangliosides are GM3 and GD3 in embryonic brains and GM1, GD1a, GD1b and GT1b in adult brains.

ceptors (mAChRs), are markedly attenuated, indicating the impairment of mAChR functions in the GM3-only mice[22], while there is no clear causal association with any aforementioned neurological abnormalities. Likewise, substantial degeneration of Purkinji neurons has also been reported in the cerebellar cortex of the double knockout mice, which may possibly result from regional complement activation and inflammatory reactions, as shown by deposits of C1q complement in the cerebella[23] and a degeneration-rescuing effect by the crossbreed carrying the disrupted gene of C3 complement[24].

A striking phenotype of high susceptibility to sound-induced seizures has been shown in another derived line of the double knockout mouse with a distinct genetic background, C57BL/6[25]. These ST-II/GalNAcT double knockout animals also display a shortened life span, typically with the death of 50% of the mice by 30 weeks of age.

Mice lacking all ganglioside expression resulting from knockout of both GalNAcT and ST-I genes suffer severe lethality. The majority of the double knockout mice die soon after weaning at 3 weeks[26]. The ganglioside-deficient mice reveal prominent vacuolization pathology in the cerebellar and spinal white matters, along with enhanced cell apoptosis, axonal degeneration and perturbed axon-glia interactions in the cerebral cortex under histopathological examinations[26].

Despite the neurological abnormalities that have been observed in ganglioside synthase knockout mice, it remains to be elucidated whether those phenotypes result from functional deficiency of the particular ganglioside product (s) and/or from an acquired consequence of the anomalous accumulation of substrate precursors.

3 Gangliosides in stem cells

Gangliosides are gaining increasing attention recently in the field of stem cell biology. Stem cells are undifferentiated cells endowed with a high potential for proliferation and the capacity for self-renewal with retention of pluripotency or multipotency to differentiate into their progenies. Stem cells have attracted considerable attention in recent years because of their biological and clinical potentials for regenerative medicine. A number of unique ganglioside markers have been identified in stem cells[27, 28]. For instance, SSEA-4 (a globo-series ganglioside having an NeuAcα2-

3Galβ1-3GalNAcβ1-R structure[29]) is specifically expressed in human pluripotent embryonic stem cells[30] and induced pluripotent stem cells[31, 32], GD3 is expressed in mouse and human mouse neural stem cells[33, 34], and GD2[34, 35] and SSEA-4[36] are expressed in human mesenchymal stem cells. Gangliosides are primarily localized on the cell surface. Thus, gangliosides can be used as specific cell surface marker molecules for identifying or isolating these stem cells[27, 28].

Also, in brain cancer stem cells, a subpopulation of brain cancer cells has been reported. These cells exhibit stem cell-like characteristics, such as the ability for self-renewal and multipotency in addition to the capability to sustain brain tumor formation. These cells also express c-series gangliosides, also known as A2B5 antigens characteristic of embryonic cells[37, 38]. These gangliosides can be utilized not only as biomarkers for cancer stem cells, but also as targets for the treatment of brain tumors. Studies of stem cell gangliosides should prove to be a fertile area of research in the future.

4 Gangliosides and diseases

Gangliosides are involved in the pathology of many diseases. For example, Guillain-Barré syndrome, an acute polyradiculoneuropathy that leads to acute quadriplegia, is caused by an autoimmune response to cell surface gangliosides[39]. In influenza, a well known viral infectious disease, influenza A viruses recognize sialic acid residues of gangliosides and glycoproteins on cell surfaces as receptor molecules for invasion of host cells[40]. Lysosomal storage diseases such as GM1 gangliosidosis and GM2 gangliosidosis (Tay-Sachs disease and Sandhoff disease) are caused by defects in the lysosomal glycosidases or specific co-activators, resulting in accumulation of the substrates, such as glycosphingolipids, including gangliosides. Human autosomal recessive infantile-onset symptomatic epilepsy syndrome, associated with developmental stagnation and blindness found in Old Order Amish pedigree, is caused by a nonsense mutation of ST-I (GM3 synthase)[41]. It has been recently suggested that GM3 in cell surface microdomains is involved in insulin resistance in type 2 diabetes, the most common metabolic disorder characterized by high blood glucose[42].

In addition, the onset of Alzheimer's disease, the most common form of dementia and neurodegenerative disease, has been proposed to be initiated by aggregation of amyloid-β peptide caused by gangliosides[43, 44]. More recently, we found an increase of Chol-1α antigens, GQ1bα and GT1aα, which are specifically expressed in cholinergic neurons[45, 46] in the brain of Alzheimer's disease model transgenic mice[47]. The increase of Chol-1α gangliosides may present evidence for generation of cholinergic neurons and neurogenesis in Alzheimer's disease brains.

Mounting evidence suggests that gangliosides modulate aggressive angiogenesis commonly found to support tumor growth. Seyfried and co-investigators[48] reported that GM3 and GD1a had an opposite effect on the responsiveness of human umbilical vein endothelial cells to vascular endothelial growth factor that promotes the endothelial cell survival, growth and migration. This is in concert with our earlier observation that GM3, a major endothelial cell ganglioside[49], was a natural angiogenesis suppressor, but GD1a, shed from the surface of certain tumor cells, could induce angiogenesis. This observation suggests that exogenously administered GM3 may have therapeutic potential for reducing angiogenesis for tumor suppression.

These above reports suggest that gangliosides are important for prevention and treatment of certain diseases. In fact, there is a series of studies showing the neurotrophic effects of intracerebroventricularly administrated GM1, which is reported to improve the cognitive function in patients with Alzheimer's disease[50].

5 Conclusion

It has been about 7 decades since Ernst Klenk, a German chemist and lipidologist (1896-1971), first isolated gangliosides from the human brain[51, 52]. Early research in the ganglioside field was focused on structural analysis of these molecules. With the advent of modern methodologies, many novel structures were identified that form the basis for further studies into gaining a better understanding of their cellular and subcellular localization in various tissue sources. This was followed by extensive investigations of their biosynthetic pathways and the regulatory mechanisms of their metabolism. Many biosynthetic and catalytic enzymes responsible for their metabolism have been characterized and glycogenes coding for these enzymes cloned and studied. These efforts have formed a firm foundation for elucidating the biological functions of these molecules. Future research should be focused on their roles not only as structural components of biomembranes, but also their functions in cell-cell recognition and adhesion, and mediators in signal transduction.

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